

What is claimed is:

1. A method to protect an animal from a disease or condition that can be treated by granulocyte colony-stimulating factor (G-CSF), comprising administering to an animal having said disease or condition a composition comprising a cysteine variant of G-CSF of SEQ ID NO: 6, wherein said G-CSF cysteine variant comprises at least one cysteine residue substituted for at least one amino acid located in at least one region of G-CSF selected from the group consisting of: the A-B loop, the B-C loop, the C-D loop, the first three or last three amino acids in helix B, the first three or last three amino acids in helix C, the first three or last three amino acids in helix D, and the amino acids following helix D.
5
10. 2. The method of Claim 1, wherein said cysteine variant comprises at least one cysteine residue substituted for at least one amino acid selected from the group consisting of: K40, S53, G55, W58, A59, P60, S62, S63, P65, S66, Q67, A68, Q70, A72, Q90, A91, E93, G94, S96, E98, G100, G125, M126,
15 A127, A129, Q131, T133, Q134, G135, A136, A139, A141, S142, A143, Q145, Q173 and P174.
20. 3. The method of Claim 1, wherein said cysteine variant comprises a cysteine residue substituted for at least one amino acid located in the C-D loop of G-CSF.
25. 4. The method of claim 3, wherein said cysteine variant comprises at least one cysteine residue substituted for at least one amino acid selected from the group consisting of G125, M126, A127, A129, Q131, Q134, G135, A136, A139, A141, and S142.
- .25 5. The method of claim 3, wherein said cysteine variant comprises a cysteine residue substituted for A141.
6. The method of claim 1, wherein said cysteine variant comprises a non-cysteine amino acid substituted for C17.
7. The method of claim 3, wherein said cysteine variant comprises a cysteine residue substituted for A141 and a non-cysteine amino acid substituted for C17.
30

8. The method of claim 1, wherein said cysteine variant is modified with a cysteine-reactive moiety.
9. The method of claim 1, wherein said cysteine variant is modified with a cysteine-reactive moiety and wherein said cysteine variant comprises a non-cysteine amino acid substituted for C17.
10. The method of claim 8, wherein said cysteine-reactive moiety is a polyethylene glycol.
11. The method of claim 9, wherein said cysteine-reactive moiety is a polyethylene glycol.
12. The method of Claim 1, wherein said composition is administered by a route selected from the group consisting of intravenous administration, intraperitoneal administration, intramuscular administration, intranodal administration, intracoronary administration, intraarterial administration, subcutaneous administration, transdermal delivery, intratracheal administration, intraarticular administration, intraventricular administration, inhalation, intranasal, intracranial, intraspinal, intraocular, aural, intranasal, oral, pulmonary administration, impregnation of a catheter, and direct injection into a tissue.
13. The method of Claim 1, wherein said composition is administered by intravenous administration.
14. The method of Claim 1, wherein said composition is administered by subcutaneous administration.
15. The method of claim 1, wherein said disease is neutropenia.
16. The method of claim 15, wherein said neutropenia is selected from the group consisting of: (a) neutropenia resulting from chemotherapy; (b) neutropenia associated with bone marrow transplantation; (c) neutropenia associated with infection with the human immunodeficiency virus; (d) neutropenia associated with burns, surgery, dilatation, anemia and neonatal septicemia; (e) severe chronic neutropenia; and (f) neutropenia associated with aplastic anemia and acute leukemia.
17. The method of claim 15, wherein said neutropenia is neutropenia resulting from chemotherapy.

18. A method to protect an animal from a disease or condition that can be
treated by erythropoietin (EPO), comprising administering to an animal having
said disease or condition a composition comprising a cysteine variant of EPO
of SEQ ID NO: 2, wherein said variant contains N24, N38, N83 and S126, and
5 wherein a cysteine residue is substituted for at least one amino acid located in
at least one region of erythropoietin selected from the group consisting of: the
region preceding helix A corresponding to amino acids 1-8 of SEQ ID NO:2,
the A - B loop corresponding to amino acids 23-58 of SEQ ID NO:2, the B - C
loop corresponding to amino acids 77-89 of SEQ ID NO:2, the C - D loop
10 corresponding to amino acids 108-131 of SEQ ID NO:2, the last three amino
acids in helix A, the first three or last three amino acids in helix B, the first
three or last three amino acids in helix C, and the first three or last three amino
acids in helix D.
19. The method of Claim 18, wherein said cysteine variant comprises a
15 cysteine residue substituted for at least one amino acid selected from the group
consisting of: A1, P2, P3, R4, D8, I25, T26, T27, G28, A30, E31, H32, S34,
N36, I39, T40, D43, T44, K45, N47, A50, K52, E55, G57, Q58, G77, Q78,
A79, S84, S85, Q86, E89, T107, R110, A111, G113, A114, Q115, K116,
E117, A118, S120, P121, P122, D123, A124, A125, A127, A128, and T132.
20. The method of Claim 18, wherein said composition is administered by
a route selected from the group consisting of intravenous administration,
intraperitoneal administration, intramuscular administration, intranodal
administration, intracoronary administration, intraarterial administration,
subcutaneous administration, transdermal delivery, intratracheal
25 administration, intraarticular administration, intraventricular administration,
inhalation, intranasal, intracranial, intraspinal, intraocular, aural, intranasal,
oral, pulmonary administration, impregnation of a catheter, and direct injection
into a tissue.
21. The method of Claim 18, wherein said composition is administered by
30 intravenous administration.
22. The method of Claim 18, wherein said composition is administered by
subcutaneous administration.

23. The method of claim 18, wherein said disease is anemia.
24. The method of claim 23, wherein said anemia is selected from the group consisting of: (a) anemia resulting from chemotherapy; (b) anemia resulting from renal disease; (c) anemia resulting from renal failure; and (d) anemia resulting from drug complications.
5
25. The method of claim 18, wherein said cysteine variant is modified with a cysteine-reactive moiety.
26. The method of claim 25, wherein said cysteine-reactive moiety is a polyethylene glycol.

10

27. A method to protect an animal from a disease or condition that can be treated by EPO, comprising administering to an animal having said disease or condition a composition comprising a cysteine variant of EPO of SEQ ID NO: 2, wherein said variant comprises a cysteine residue substituted for at least one amino acid selected from the group consisting of: A1, P2, P3, R4, D8, I25, T26, T27, G28, A30, E31, H32, S34, N36, I39, T40, D43, T44, K45, N47, A50, K52, E55, G57, Q58, G77, Q78, A79, S84, S85, Q86, E89, T107, R110, A111, G113, A114, Q115, K116, E117, A118, S120, P121, P122, D123, A124, A125, A127, A128, T132, K154, T157, G158, E159, A160, T163, G164, and D165.
- 5
28. The method of Claim 27, wherein said composition is administered by a route selected from the group consisting of intravenous administration, intraperitoneal administration, intramuscular administration, intranodal administration, intracoronary administration, intraarterial administration, 15 subcutaneous administration, transdermal delivery, intratracheal administration, intraarticular administration, intraventricular administration, inhalation, intranasal, intracranial, intraspinal, intraocular, aural, intranasal, oral, pulmonary administration, impregnation of a catheter, and direct injection into a tissue.
- 20
29. The method of Claim 27, wherein said composition is administered by intravenous administration.
30. The method of Claim 27, wherein said composition is administered by subcutaneous administration.
31. The method of claim 27, wherein said disease is anemia.
- 25
32. The method of claim 31, wherein said anemia is selected from the group consisting of: (a) anemia resulting from chemotherapy; (b) anemia resulting from renal disease; (c) anemia resulting from renal failure; and (d) anemia resulting from drug complications..
33. The method of claim 27, wherein said cysteine variant is modified with a cysteine-reactive moiety.
- 30
34. The method of claim 33, wherein said cysteine-reactive moiety is a polyethylene glycol.

35. A method to protect an animal from a disease or condition that can be treated by EPO, comprising administering to an animal having said disease or condition a composition comprising a cysteine variant of EPO of SEQ ID NO: 2, wherein said variant comprises at least one cysteine residue added preceding the first amino acid of the mature protein or following the last amino acid of the protein.
- 5
36. The method of claim 35, wherein said cysteine variant comprises at least one cysteine residue added following R166.
- 10
37. The method of Claim 35 wherein said composition is administered by a route selected from the group consisting of intravenous administration, intraperitoneal administration, intramuscular administration, intranodal administration, intracoronary administration, intraarterial administration, subcutaneous administration, transdermal delivery, intratracheal administration, intraarticular administration, intraventricular administration, 15 inhalation, intranasal, intracranial, intraspinal, intraocular, aural, intranasal, oral, pulmonary administration, impregnation of a catheter, and direct injection into a tissue.
- 15
38. The method of Claim 35, wherein said composition is administered by intravenous administration.
- 20
39. The method of Claim 35, wherein said composition is administered by subcutaneous administration.
40. The method of claim 35, wherein said disease is anemia.
41. The method of claim 40, wherein said anemia is selected from the group consisting of: (a) anemia resulting from chemotherapy; (b) anemia resulting from renal disease; (c) anemia resulting from renal failure; and (d) anemia resulting from drug complications.
- 25
42. The method of claim 35, wherein said cysteine variant is modified with a cysteine-reactive moiety.
43. The method of claim 42, wherein said cysteine-reactive moiety is a polyethylene glycol.
- 30

44. A method to protect an animal from a disease or condition that can be treated by alpha interferon, comprising administering to an animal having said disease or condition a composition comprising a cysteine variant of alpha interferon-2 of SEQ ID NO: 3, wherein said alpha interferon-2 cysteine variant comprises at least one cysteine residue substituted for at least one amino acid located in at least one region of alpha interferon-2 selected from the group consisting of: the B - C loop, the C - D loop, the D-E loop, the first three or last three amino acids in helix A, the first three or last three amino acids in helix B, the first three or last three amino acids in helix C, the first three or last three amino acids in helix D, the first three or last three amino acids in helix E, the amino acids preceding helix A, and the amino acids following helix E.
- 5
- 10
45. The method of Claim 44, wherein said cysteine variant comprises at least one cysteine residue substituted for an amino acid selected from the group consisting of: D2, L3, P4, Q5, T6, S8, Q20, S73, A74, A75, D77, E78, T79, Q101, G102, G104, T106, E107, T108, P109, M111, K112, E113, D114, S115, K131, E132, K133, K134, Y135, S136, A139, S152, S154, T155, N156, L157, Q158, E159, S160, L161, R162, S163, K164, and E165.
- 15
46. The method of claim 44, wherein said cysteine variant comprises at least one cysteine residue substituted for at least one amino acid located in the region of alpha interferon-2 preceding helix A.
- 20
47. The method of claim 46, wherein said cysteine variant comprises at least one cysteine residue substituted for at least one amino acid selected from the group consisting of D2, L3, P4, Q5, T6, and S8.
48. The method of Claim 46, wherein said cysteine variant comprises a cysteine residue substituted for Q5.
- 25
49. The method of claim 44, wherein said cysteine variant comprises at least one cysteine residue substituted for at least one amino acid located in the C – D loop of alpha interferon-2.
50. The method of claim 49, wherein said cysteine variant comprises at least one cysteine residue substituted for at least one amino acid selected from the group consisting of Q101, G102, G104, T106, E107, T108, and P109.
- 30

51. The method of Claim 49, wherein said cysteine variant comprises a cysteine residue substituted for M111.
52. The method of Claim 44, wherein said composition is administered by a route selected from the group consisting of intravenous administration, intraperitoneal administration, intramuscular administration, intranodal administration, intracoronary administration, intraarterial administration, subcutaneous administration, transdermal delivery, intratracheal administration, intraarticular administration, intraventricular administration, inhalation, intranasal, intracranial, intraspinal, intraocular, aural, intranasal, oral, pulmonary administration, impregnation of a catheter, and direct injection into a tissue.
10
53. The method of Claim 44, wherein said composition is administered by intravenous administration.
54. The method of Claim 44, wherein said composition is administered by subcutaneous administration.
15
55. The method of claim 44, wherein said cysteine variant of alpha interferon-2 is modified with a cysteine-reactive moiety.
56. The method of claim 55, wherein said cysteine reactive moiety is a polyethylene glycol.
20
57. The method of claim 44, wherein said disease is cancer.
58. The method of claim 44, wherein said disease is a viral disease.
59. The method of claim 58, wherein said viral disease is selected from the group consisting of Hepatitis B and Hepatitis C.
25